

Spectrophotometric study of complexation equilibria with H-point standard addition and H-point curve isolation methods

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Abstract

The use of H-point curve isolation (HPCIM) and H-point standard addition methods (HPSAM) for spectrophotometric studies of complex formation equilibria are proposed. One step complex formation, two successive stepwise and mononuclear complex formation systems, and competitive complexation systems are studied successfully by the proposed methods. HPCIM is used for extracting the spectrum of complex or sum of complex species and HPSAM is used for calculation of equilibrium concentrations of ligand for each sample. The outputs of these procedures are complete concentration profiles of equilibrium system, spectral profile of intermediate components, and good estimation of conditional formation constants. The reliability of the method is evaluated using model data. Spectrophotometric studies of murexide–calcium, dithizone–nickel, methyl thymol blue (MTB)–copper, and competition of murexide and sulfate ions for complexation with zinc, are used as experimental model systems with different complexation stoichiometries and spectral overlapping of involved components.

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1. Introduction

Spectrophotometric methods are, in general, highly sensitive, and as such are suitable for studying chemical equilibria in solution. When the components involved in the chemical equilibrium have distinct spectral responses, their concentrations can be measured directly, and the determination of equilibrium constant is trivial. However, in many cases, the spectral responses of two and sometimes even more components overlap considerably, and analysis is no longer straightforward. Several spectrophotometric methods have been developed to determine the equilibrium constants of chemical processes. Occasionally, problems arise because of strong overlapping of chemical components involved in equilibrium and uncertainties from using some complex mathematical algorithms, to solve such problems [1–3].

Several univariate least squares curve fitting methods have been traditionally proposed to interpret the experimental data [4,5] and they have shown their ability to determine the stoichiometric and formation constants. Developments in the

field of computation of equilibrium constants from experimental data were reviewed a few years ago [6–9], and description of most used computer programs for evaluation of stability constants from spectrophotometric data based on knowledge of chemical model, is considered. Recently too, applications of derivative spectrophotometric method for studying the chemical equilibria are reported, which is limited to quantitative analysis of only two component systems [10–12].

However, much more information can be extracted if multivariate spectroscopic data are analyzed by means of an appropriate multivariate data analysis method. On the other hand, equilibrium transformations, especially complexation ones, sometimes involve minor changes in their electronic spectra, which makes it impossible to employ classical spectrophotometry for determination of equilibrium constants [13].

The proper chemometric algorithms can be used for evaluating the equilibrium information such as the stability constant through analysis of spectroscopic data. Several soft-modeling and hard-modeling algorithms have been developed that analyze bilinear data obtained from chemical systems. Soft-modeling methods range from very general approaches with minimal demands on the structure of data,

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such as EFA [14], HELP [15], SIMPLISMA [16], and ALS [17], to methods which rely on trilinearity, such as PARAFAC [18], GRAM [19], or TLD [20]. Hard-modeling approaches of fitting multivariate response data are based on mathematical relationships, which describe the measurements quantitatively [21,22]. In chemical equilibria, the analysis is based on the equilibrium model which quantitatively describes the reaction and all concentrations in the solution under investigation.

The H-point curve isolation method (HPCIM) was proposed [23,24] for analysis of a binary mixture with known component *X* and unknown one *Y*. With this method, it is possible to plot the spectrum of *Y* species in a simple way, without forcing the data to fit a specific model. It is only necessary to have the analyte and the sample spectra. The method cancels the contribution of *X* in the sample signal, thereby giving a set of possible spectra for the *Y* species. From this set, the real *Y* spectrum can be calculated by finding pairs of wavelengths according to the calibration model of the H-point standard addition method (HPSAM) [25,26]. The HPSAM analytical signal (absorbance increment at two wavelengths with the same absorbance value for the *Y* component) is only dependent on one species because, at each point, the absorbances of the other compounds present in the sample are cancelled. If matrix effects are absent, standard addition is not needed and molar absorption coefficients can be used in the HPSAM equation. HPSAM and HPCIM are frequently used for simultaneous analysis of analyte mixtures by strong or even full spectral overlapping of components [27–33]. However, less attention has been paid to application of the methods in the studies of chemical equilibria [34]. To the best of our knowledge, this is the first application of HPSAM and HPCIM for spectrophotometric studies of complexation equilibria. Although the methods have been used successfully for quantitative analysis, the feasibility of HPSAM methods for studying complexation equilibria are not considered so far. The proposed method is comparable to hard modeling method for complete resolving of some chemical system, without requiring the application of curve fitting programs.

In this work, a method for spectrophotometric study of direct and competitive metal ion–ligand complex formation is described. H-point standard addition method is used for the determination of equilibrium concentration of ligand at each mole ratio and then, the stability of complex formed is estimated. Also, a simultaneous application of HPCIM and HPSAM has been presented for studying the stepwise complex formation. HPCIM is used for omitting the spectrum of ligand and HPSAM for calculation of the equilibrium free ligand concentrations.

2. Experimental

UV-Vis absorbance digitized spectra were collected using a CARY5 spectrophotometer, 1 cm quartz cells, at a

scan rate of 100 nm min⁻¹ and a slit width of 2 mm. The recorded spectra were digitized with one data point per nanometer. Measurements of pH were made with Metrohm 713 pH-meter using a combined glass electrode. Murexide, dithizone, methyl thymol blue (MTB), and all metal ion salts were purchased from Merck and used without further purification. All of the solutions were prepared fresh daily. Solutions were allowed to remain in a thermostated sample compartment for minimum of 10 min before the spectra were collected. The temperature was maintained at 25.0 ± 0.1 °C using a Fisher Scientific Isotemp constant temperature circulator. Specific details are given in Section 4.

3. Theoretical background

3.1. One step complex formation

The conditional complex formation between metal ion *M*, and ligand *L* in one step, is defined by the chemical equilibrium



The corresponding conditional complex formation constant K_f , is

$$K_f = \frac{[ML_n]}{[M][L]^n} \quad (2)$$

where $[L]$, $[M]$, and $[ML_n]$ are equilibrium concentrations of all forms of ligand, metal ion, and the complex under conditions of experiment, respectively. The mass balances of the system in different mole ratios of metal ion to ligand can be written as

$$C_L = [L] + n[ML_n] \quad (3)$$

$$C_M = [M] + [ML_n] \quad (4)$$

C_L is the total concentration of ligand, which remains constant, and C_M is the total concentration of metal ion, which varies on employing the mole ratio method. At zero mole ratio, the spectrum recorded is the pure spectrum of the concentration of ligand C_L and in the presence of excess metal ion for nearly strong complexation reaction (the level off region of mole ratio plot), the obtained spectrum is the pure spectrum of the concentration of complex C_L/n . It is clear that n can be determined according to mole ratio plot [35] or applying continuous variation method [36–38]. So, in the spectral region where the ligand and complex have strong spectral overlapping, each solution at a particular mole ratio can be considered as a mixture of two components with known absorption spectra. In this step, by considering the spectrum of metal complex ML_n , two wavelengths are selected according to HPSAM criteria [26] and the equilibrium concentration of ligand is calculated from

$$-C_H = [L] = \frac{A_2 - A_1}{M_1 - M_2} \quad (5)$$

C_H is the equilibrium concentration of ligand, $A_2 - A_1$ is the absorbance increment for analyzing certain mixtures at a particular mole ratio and $M_1 - M_2$ is the slope increment for pure ligand standard solutions at wavelength pairs selected. The calculated values of $[L]$ according to Eq. (5) in each mole ratio produce the concentration profile of ligand in the evolutionary process. Once $[L]$ is known, the equilibrium concentration of remaining species can be easily computed from the Eqs. (3) and (4).

3.2. Two successive stepwise complex formation

The two successive stepwise complex formations are defined by the chemical equilibria



The corresponding stepwise complex conditional formation constants are

$$K_1 = \frac{[ML]}{[M][L]} \quad (8)$$

$$K_2 = \frac{[ML_2]}{[ML][L]} \quad (9)$$

The mass balances of the system in different mole ratios of metal ion to ligand can be written as

$$C_L = [L] + [ML] + 2[ML_2] \quad (10)$$

$$C_M = [M] + [ML] + [ML_2] \quad (11)$$

At mole ratio equal to zero, the recorded spectrum is the pure spectrum of concentration of ligand C_L and in the presence of excess metal ion for nearly strong complex formation reactions (the level off region of mole ratio plot) the obtained spectra is the pure spectrum of concentration of ML complex C_L/n . So, in the spectral region where ligand and the two complexes have a strong spectral overlapping, each solution at a particular mole ratio can be considered as a mixture solution of three components with known absorption spectra of two components (L and ML). In this case, analysis of the mixture is possible according to simultaneous application of H-point curve isolation and H-point standard addition methods.

In each solution at a particular mole ratio, absorbance at every wavelength will be the sum of absorbances of the L, ML, and ML_2 , so it can be written as follows:

$$s_i = l_i + c_i \quad (12)$$

where s_i is the absorbance of the mixture solution at the i th wavelength, the subscript i refers to the absorbance at a certain wavelength in the $[i, n]$ range, and l_i and c_i are the absorbances of ligand and sum of both complexes, respectively. According to HPCIM (as presented in Appendix A), it is possible to cancel the contribution of the L signal in the sample signal, thereby giving a set of possible spectra

for the sum of both complexes. From this set, the real c_i spectrum can be calculated by finding pairs of wavelengths according to the calibration model of the H-point standard addition method. Applying the HPCIM procedure again on c_i , spectrum calculated in mole ratio of 0.5, leads to canceling the contribution of ML spectrum and obtaining the ML_2 spectrum. Now, the spectrum monitored at each mole ratio s_i , can be considered as the spectrum of a ternary mixture (L, ML, and ML_2) with known spectra of all components, and so, the special version of HPSAM for analyte determination in ternary mixture [39] (Appendix B) can be applied for calculation of concentration profile of L and ML species. The concentration of each component (L or ML) was calculated from spectra overlapping at two appropriate wavelengths, when the other two species were selected as interferences present in the equal absorbance relationship.

3.3. Competitive complex formation

When two ligands, which can form complexes with the same metal ion, are present together in solution, the extent of complexation with each ligand is determined by their concentrations and the respective metal–ligand stability constants. If the stability constant and the concentration of species associated with one ligand in mixed solution are known, the stability constant with the other ligand can be calculated, even if only the concentration of the second ligand is known. The method of competitive equilibration is clearly a potentially useful method for measuring formation constants of ligands that are otherwise difficult or impossible to obtain, e.g. the environmental mobility of radionuclides can be strongly influenced by the presence of naturally occurring ligands such as humates and fulvates, the stability constants of which are difficult to measure by other methods [40–42].

Suppose the reactions in which two ligands L and L' compete for the same metal ion, are as follows (the chemical components involved in complexation are considered as formal forms and the charges are omitted for simplicity)



The reaction ($M + L$) is a colored reaction and the other reaction ($M + L'$) is a colorless reaction in a competitive system. The mass balances of the system in different concentrations of metal ions and fixed concentration of ligand can be written as

$$C_L = [L] + [ML] \quad (15)$$

$$C_{L'} = [L'] + [ML'] \quad (16)$$

$$C_M = [M] + [ML] + [ML'] \quad (17)$$

C_L and $C_{L'}$ are total concentrations of both ligands, which remain constant, and C_M is the total concentration of metal ion, which varies in the process. According to the proposed

method presented in Section 3.1, the concentration profile of L in the presence and absence of competitive ligand can be calculated. The stability constant obtained from concentration profiles derived in the absence of L' can be used for obtaining the concentration profiles of all chemical species in the competitive system by considering Eqs. (13)–(17).

4. Results and discussion

4.1. Simulation

To demonstrate the ability of the proposed method for obtaining the concentration profiles of species involved in one step complexation equilibrium, two spectra were created and summed together in known proportion chosen to mimic model of mole ratio method. The two curves, represent model ligand and complex spectra, respectively. Simulated spectra of species were produced by gaussian function. Random error was added to the set of artificial data generated to test the method more rigorously. The error is a set of noise in agreement with the gaussian distribution with mean zero and standard deviation of 0.004. A model based on mole ratio method was used to calculate the concentration profiles of ligand and complex species and thus, weigh the contribution of each pure spectrum in the total spectrum generated using a multicomponent Beer's law expression. The total concentration of ligand was kept constant in the creation of the synthesized spectral data, since, our experimental data are collected under this condition. A polynomial equation, which results from the combination of the formation constant and the mass balance for the metal ion and the ligand, can be used for calculating the ligand concentration profile. The combination of Eqs. (2)–(4) as a function of the free ligand concentration [L], yields the expression:

$$K_f[L]^{n+1} + (nK_fC_M - K_fC_L)[L]^n + [L] - C_L = 0 \quad (18)$$

If the values of n , C_M , C_L and K_f are known, it is possible to obtain the free ligand concentration [L] from the roots of the associated polynomial. This can be done numerically by the command *roots* of MATLAB [43]. It then becomes a question of selecting the proper value of [L] from the non-complex and non-negative roots (usually it is the minimum one). Once [L] is known, the equilibrium concentration of remaining species can be easily computed from the combination of Eqs. (2)–(4), which yields:

$$[M] = \frac{C_M}{(1 + K_f[L]^n)} \quad (19)$$

$$[ML_n] = \frac{K_fC_M[L]^n}{(1 + K_f[L]^n)} \quad (20)$$

The model data sets are based on $n = 1$ and 2 being studied. It is simplified to make the data set related to a real system. However, the method is applicable to any one-step complexation system for which n is known. In creating the

data sets, the $K_f = 1.0 \times 10^5$ (for $n = 1$) and $K_f = 1.0 \times 10^{10}$ (for $n = 2$) were used. The generated spectra corresponded to mole ratio ranging from 0 to 2.

Also, to evaluate the performance of the method, a set of artificial data was created according to mole ratio method, for studying the two successive stepwise and mononuclear complex formation systems. A polynomial equation which results from the combination of the formation constant and the mass balance for the metal and the ligand can be used for calculating the ligand concentration profile. The combination of Eqs. (8)–(11) as a function of the free ligand concentration [L] yields the expression:

$$K_1K_2[L]^3 + (K_1 + 2K_1K_2C_M - K_1K_2C_L)[L]^2 + (1 + K_1C_M - K_1C_L)[L] - C_L = 0 \quad (21)$$

Once the concentration profile of ligand was calculated for certain K_1 and K_2 values by using MATLAB command *roots*, the equilibrium concentration of remaining species can be easily computed from the combination of Eqs. (8)–(11). In creating the data sets, the values $K_1 = 1.0 \times 10^6$ and $K_2 = 1.0 \times 10^4$ were used. The generated spectra corresponded to mole ratio ranging from 0 to 2.

4.2. Resolving the simulated and real data

Fig. 1 shows absorption spectra created for two hypothetical one step 1:1 and 1:2 metal–ligand complex formation systems, at fixed concentration of ligand and varied concentrations of metal ions. The constructed model showed that, in the range of reliable formation constants, the pure spectrum of complex species could be obtained in the presence of excess amounts of metal ion. However, for small formation constants, for which obtaining the pure spectrum of complex species is not possible experimentally, the HPCIM can be used for extracting them from mixed spectrum measured at high mole ratio points (Appendix A) [23,24]. The proposed method based on HPSAM was applied by using the artificial data sets of two above-mentioned systems. As shown in Fig. 2, the data points in the figure denote the concentration profiles calculated according to HPSAM method, while the lines represent the given values. It is apparent that the two data sets for each system considered are in good agreement with each other. The calculated equilibrium concentrations of all species at each mole ratio can be used for appropriate estimation of equilibrium formation constants. The solved and known concentration profiles are in good accordance as well. From the above results, it can be concluded that the HPSAM can successfully predict the concentration profiles of each component and equilibrium parameters.

The proposed spectrophotometric H-point standard addition method was confirmed by complexation study of two different chemical systems. The one step complex formation in murexide–calcium [44] and dithizone–nickel [44] systems were selected as a model for 1:1 and 2:1 complexation equilibria with strong spectral overlapping between

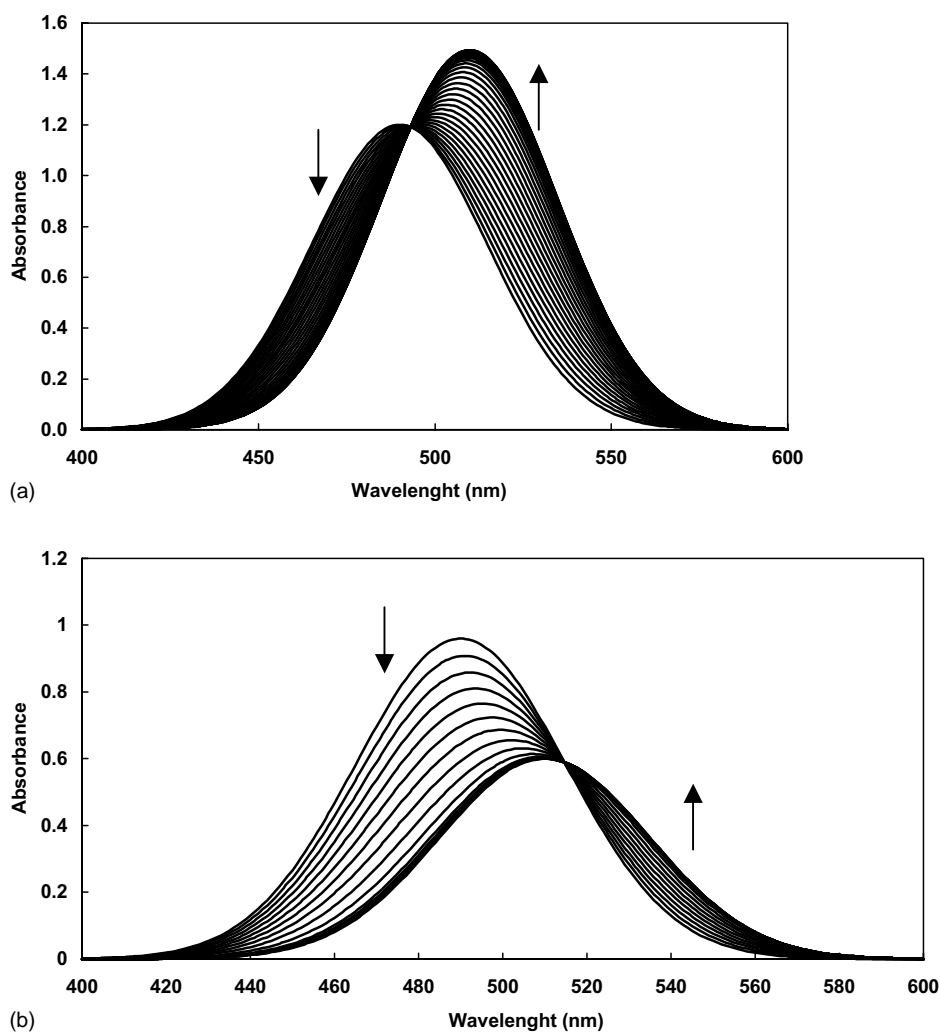


Fig. 1. Simulated absorption spectra of two hypothetical one step (a) 1:1 and (b) 1:2 metal–ligand complex formation systems, at fixed concentration of ligands and varied concentrations of metal ions.

two components of chemical equilibria. Mole ratio method was used and the spectra of solutions containing a constant amount of the ligand at fixed pH and varying amounts of metal ions were obtained. Fig. 3a and b show the typical evolutionary process of complexation for murexide–Ca (pH 12) and dithizone–Ni systems (pH 7), respectively. A clear isosbestic point in each figure indicate the occurrence of one step complex formation during the titration of ligands with metal ions.

In dithizone–nickel system, the HPCIM is used for extracting the spectrum of 2:1 complex from mixed spectrum measured at high mole ratio points (Appendix A) and in murexide–calcium system, the spectrum obtained in the presence of excess metal ions is considered as the spectrum of nearly pure complex. It is possible to select several pairs of wavelengths which present the same absorbance for this component. After this, the selected wavelength pairs are sorted following the criteria of giving the higher value in the difference of slopes of calibration lines for pure standard

ligand solutions. The equation that is used for calculation of C_H (Eq. (5)) can be seen as a ratio between an absorbance increment (ΔA) and a slope increment (ΔM). As shown previously by Campins-Falco and coworkers [26], the higher the value for the slope increment (ΔM), the lower the error for the analyte concentration. So, only the wavelength pair that gives greatest slope increment value was selected. According to the characteristics of HPSAM at H-point, C_H (concentration of free ligand) is independent of the concentration of another species considered (metal complex).

Concentration of free ligand is calculated based on HPSAM equation (Eq. (5)) for two systems and related mole ratio points. According to mass balances of the systems considered (Eqs. (3) and (4)), equilibrium concentrations of metal ions and complexes are also calculated. Fig. 4a and b show the concentration profiles obtained for murexide–Ca and dithiazone–Ni systems, correspondingly. By inserting the values of variables into Eq. (2), the equilibrium constants were determined. When calculating the equilibrium

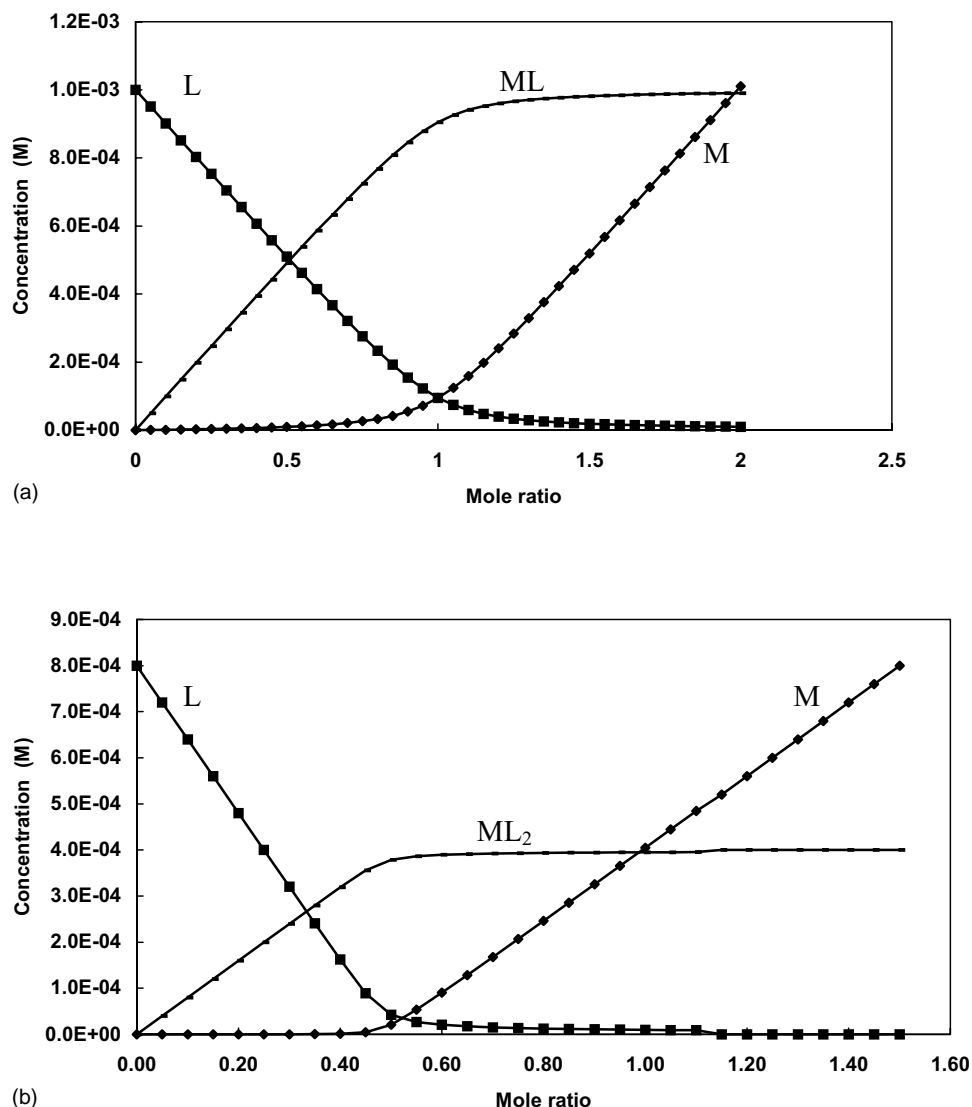


Fig. 2. Concentration profiles obtained by proposed method on the spectra of the two systems shown in Fig. 1. The data points denote the concentration profiles calculated according to HPSAM method, while the lines present the given values.

constants, care must be taken to consider only the feasible region for each constant [45]. The best region for determining the equilibrium constant corresponds to the mole ratio where the concentrations of the equilibrium species are nearly equal. This situation occurs near the intersection

of the profiles shown in Fig. 4. The logarithm of the conditional formation constants, and their means and standard deviations, calculated in this way are listed in Table 1.

Fig. 5 shows absorption spectra created for 1:1 and 1:2 metal–ligand stepwise complex formation systems, at fixed

Table 1

Logarithm of equilibrium conditional formation constants calculated from the concentration profiles obtained from the proposed method

Considered model	Simulated system		Complexes	Real system	
	Given	Calculated		Calculated	Reported ^a
M + L	5.0	5.0 ± 0.0	Murexide–Ca	5.46 ± 0.20	5.89 ± 0.01
M + 2L	10.0	10.01 ± 0.03	Dithizone–Ni	10.30 ± 0.41	10.16 ± 0.01
M + L	6.0	5.83 ± 0.54	MTB–Cu	5.30 ± 0.63	6.25 ± 0.01
ML + L	4.0	3.99 ± 0.02		3.92 ± 0.20	4.13 ± 0.02
M + L	5.0	5.0 ± 0.1	Murexide–Zn	5.02 ± 0.01	–
M + L'	0.5	0.5 ± 0.01	Sulfate–Zn	3.43 ± 0.01	–

^a According to [46].

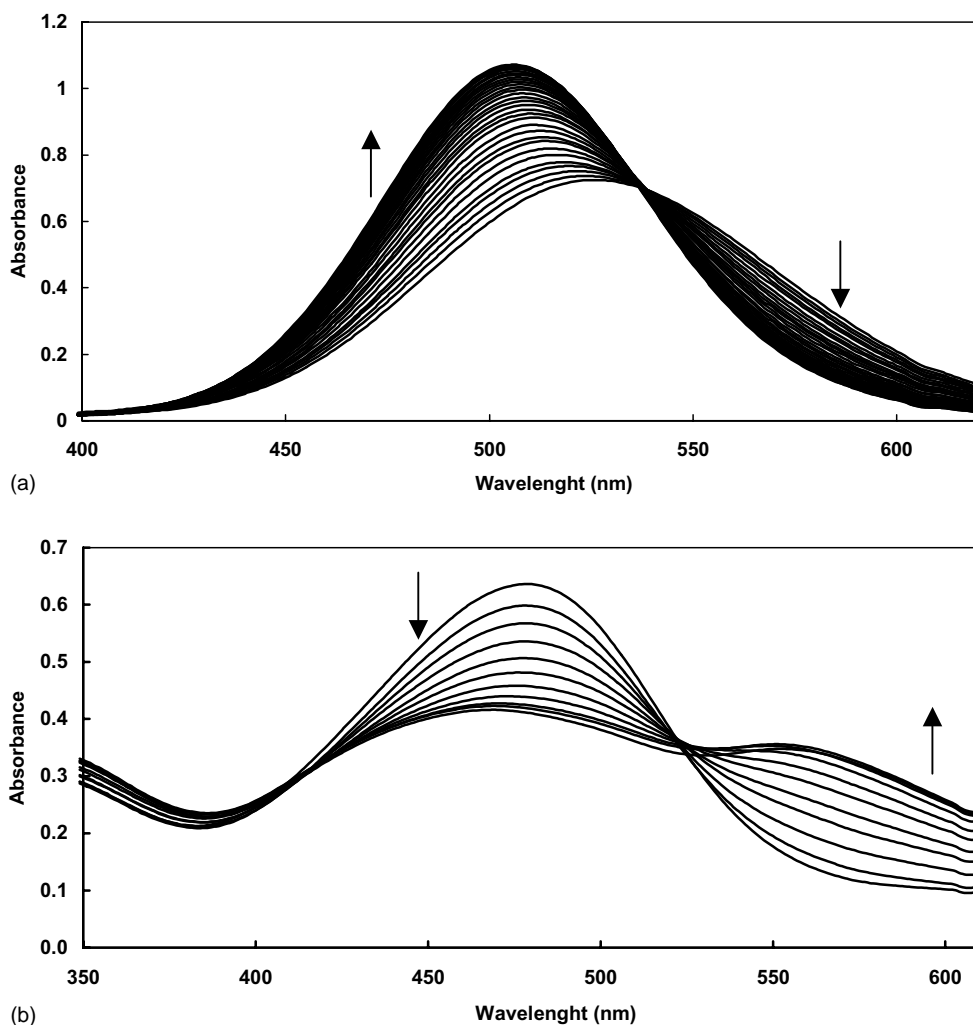


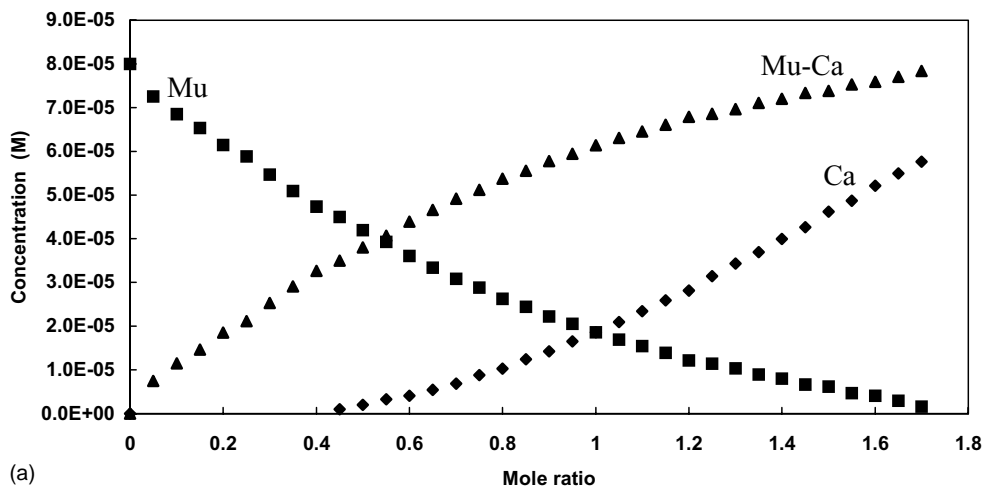
Fig. 3. Experimental absorption spectra of (a) murexide–Ca system at 8.0×10^{-5} M murexide and various concentrations of Ca^{2+} at pH 12 and (b) dithiazone–Ni system at 4.7×10^{-5} M dithiazone and various concentrations of Ni^{2+} at pH 7.

concentration of ligand and varied concentrations of metal ions. The proposed method based on applying HPCIM and HPSAM successively, was used to analyse the artificial data set. Fig. 6 shows a good agreement between the data points (the concentration profile calculated according to proposed method) and the line (given values). The calculated equilibrium concentrations of all species at each mole ratio can be used for appropriate estimation of equilibrium formation constants (Table 1). The solved and known concentration profiles are in good accordance as well. From the above results, it can be concluded that the proposed method can successfully predict the concentration profiles of each component and equilibrium parameters.

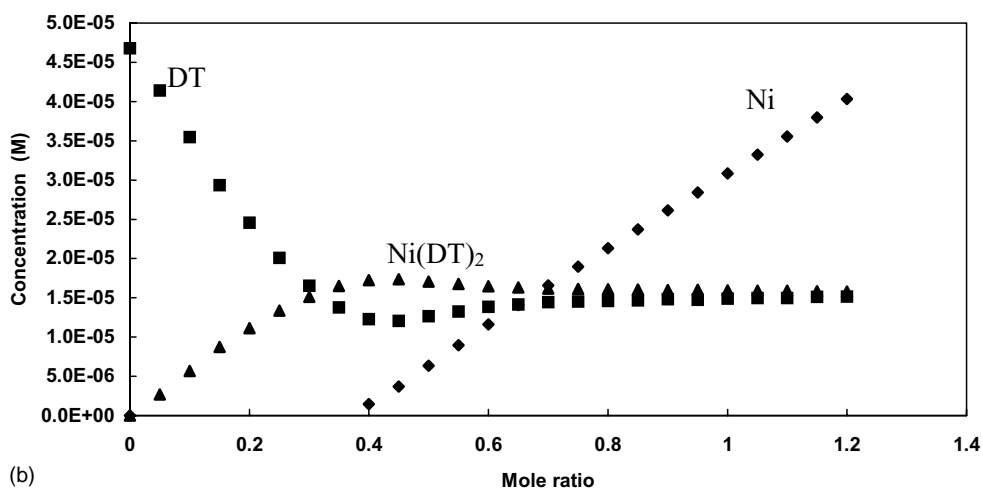
The favorable results obtained with the artificial test data clearly showed that the approach to computing the equilibrium concentration profiles in two successive stepwise complex formation, is sufficiently accurate for analyzing real spectral data, and thus estimating the stepwise formation constants. Fig. 7 shows the absorption spectra of a series of solutions containing a constant concentration of MTB at

fixed pH of 5, and varying amounts of Cu^{2+} . The spectra indicate that Cu^{2+} can form two complexes of 1:1 and 1:2 (metal-to-ligand) stoichiometry with MTB. While MTB absorbs light at 435 nm, maximum absorption wavelengths for 1:1 and 1:2 complexes are located at about 596 and 499 nm, respectively. In addition to this, there are two clear isobestic points in the corresponding spectra indicating the occurrence of two consecutive equilibria during the evolving process. As mentioned above, performing the HPCIM two times on the spectrum at a mole ratio of 0.5, can be used for obtaining the spectrum of ML_2 component and performing HPSAM for ternary mixture analysis, is used for calculation of ML and L equilibrium concentration profiles.

The spectrum measured at a mole ratio of 0.5 can be used for deriving the spectrum of 1:2 complex by employing the HPCIM twice. In the first step, the contribution of the ligand and in the second step, the contribution of the 1:1 complex is cancelled. Considering the calculated spectrum for ML_2 and known spectra of L and ML, the application of HPSAM is possible. If ML and ML_2 are the interferents, then the two



(a)



(b)

Fig. 4. Concentration profiles obtained for (a) murexide–Ca and (b) dithizone–Ni systems.

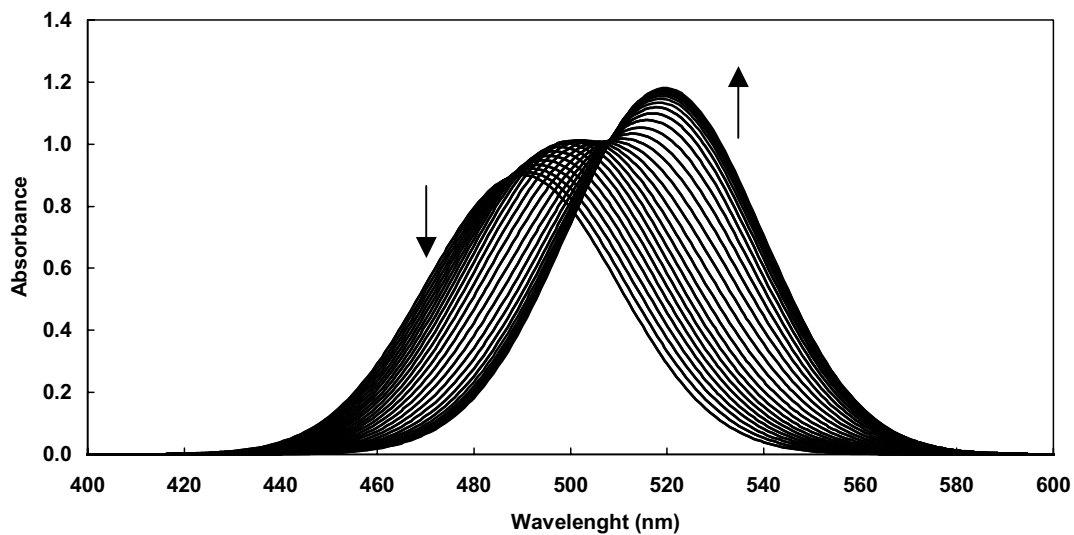


Fig. 5. Simulated absorption spectra of hypothetical two successive stepwise 1:1 and 1:2 metal–ligand complex formation systems at fixed concentration of ligands and varied concentrations of metal ions.

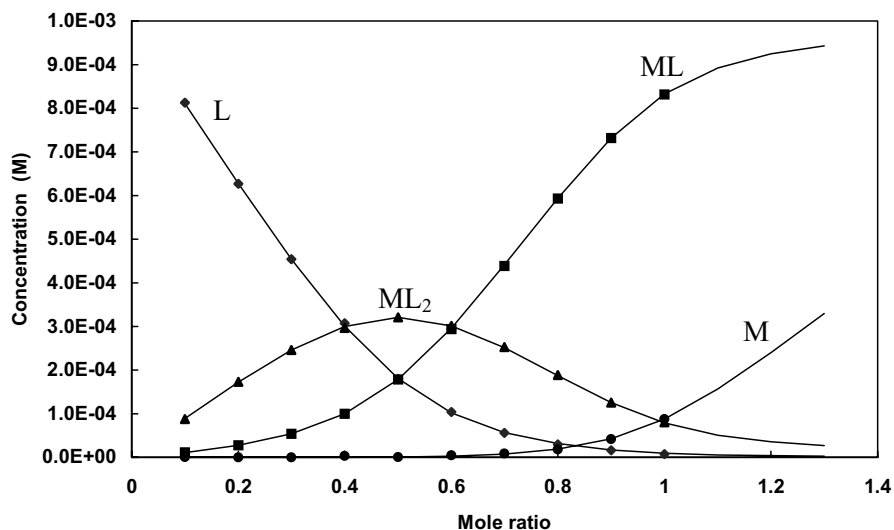


Fig. 6. Concentration profiles obtained by the proposed method on the simulated spectra of two step 1:1 and 1:2 metal–ligand complex formation systems shown in Fig. 5. The data points denote the concentration profiles calculated according to the proposed method, while the lines present the given values.

analytical wavelengths should be consistent with Eq. (B.5) (see Appendix B). It can be seen that we can obtain the wavelength pairs that give the same value from the quotient spectra between ML and ML_2 , and then calculate the concentration of L. Fig. 8 shows the quotient spectra between each of the two species of ML_2 , L, and ML. It is apparent that there are many wavelength pairs that can be selected from each quotient spectrum. Better results are obtained if the wavelength pair selected, corresponds to a rather larger value of the quotient so that the similarity between the two interferences is minimized, and if the absolute value of denominator of Eq. (B.5) is also relatively large. In addition, the absorbance at the selected wavelengths should be as large as possible in order to reduce the measurement errors. According to these points, analytical wavelength pairs 383/455 and 433/563 nm were selected for ML/ ML_2 and L/ ML_2 , respectively.

The concentration profiles of L and ML compounds were calculated from spectra measured in each mole ratio at two appropriate selected wavelength pairs based on HPSAM equation (Eq. (B.5)). Finally by using the mass balances (Eqs. (13) and (14)), the concentration profiles of other chemical species (M, ML_2) were calculated. Fig. 9 shows the concentration profiles obtained for MTB–Cu system. The estimated conditional stepwise formation constants are presented in Table 1. We calculated these conditional formation constants in another work [46], by applying a more complex chemometrics method based on rank annihilation factor analysis which are presented in Table 1. The good agreement between calculated stability parameters by the presented method based on HPCIM–HPSAM and our other work is an evidence of the ability and comparability of the proposed method, with precise complex chemometrics method based on principal component analysis.

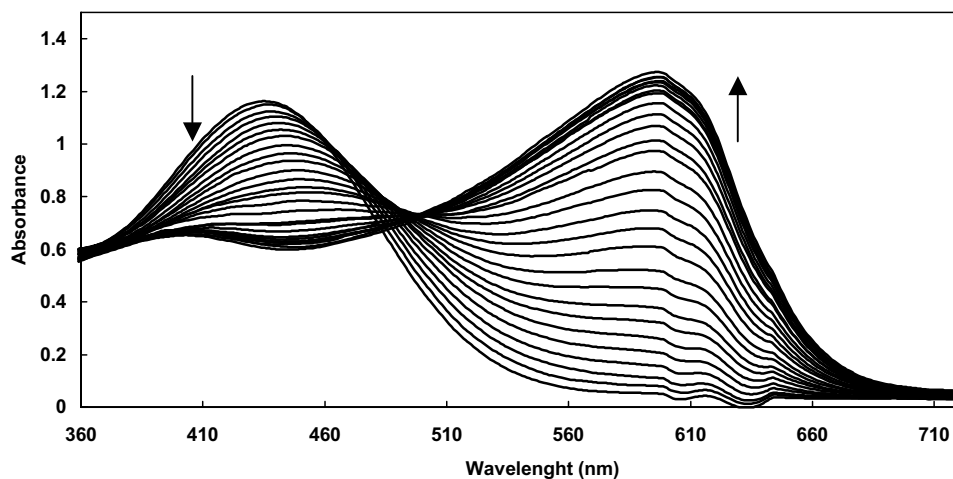
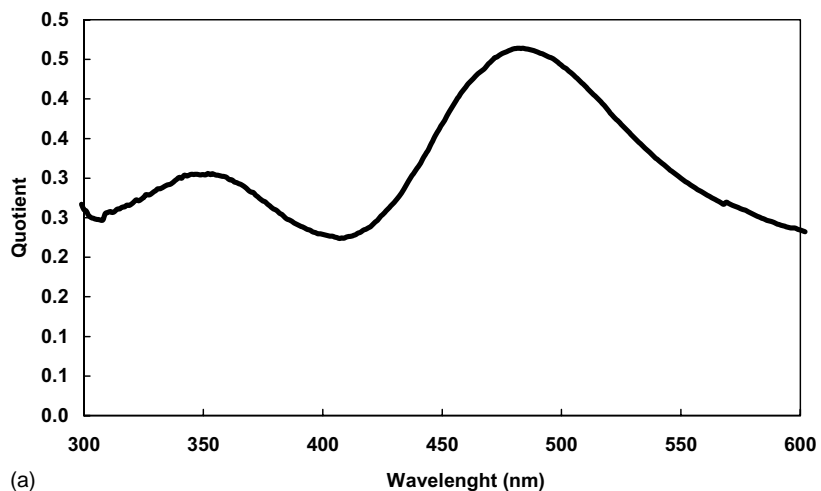
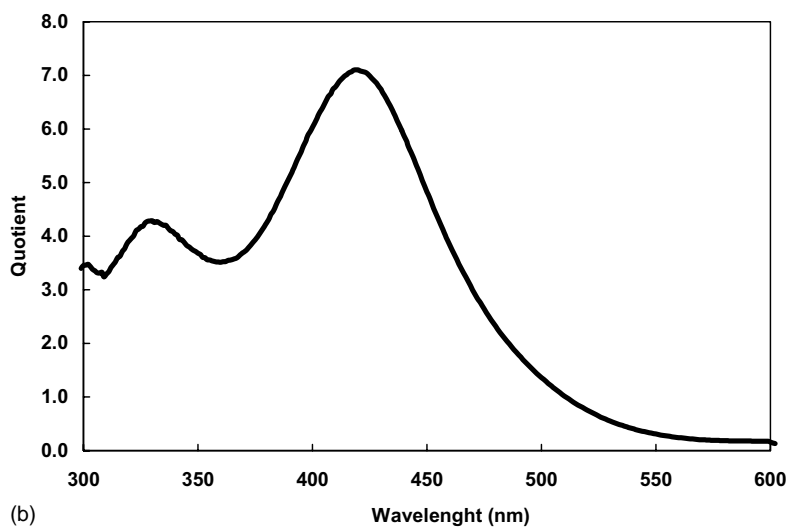


Fig. 7. Experimental absorption spectra of a series of solution containing a constant concentration (1.6×10^{-4} M) of the methyl thymol blue at fixed pH of 5.0 and varying amounts of Cu^{2+} .



(a)



(b)

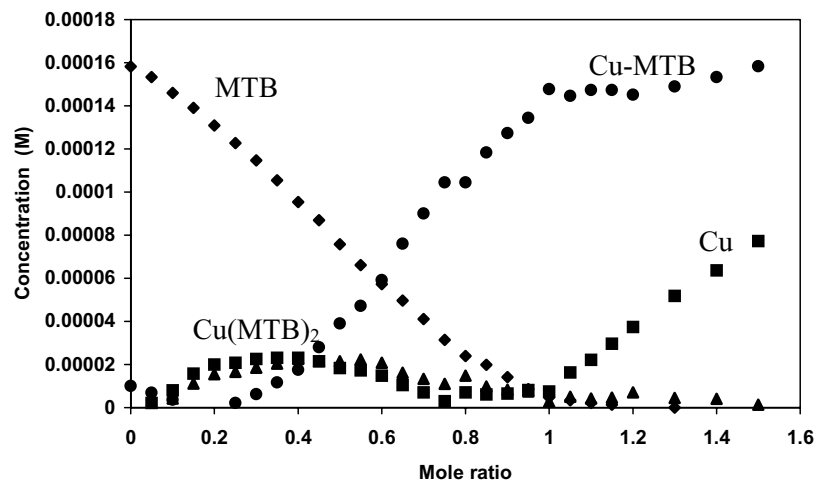
Fig. 8. The quotient spectra between two species (a) ML_2 to L and (b) ML_2 to ML.

Fig. 9. Concentration profiles obtained for MTB–Cu system according to the proposed method.

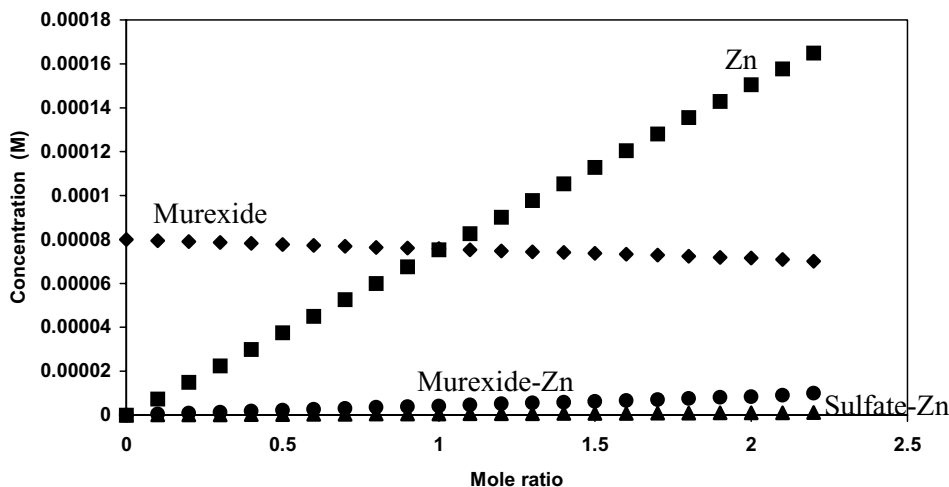


Fig. 10. Concentration profiles obtained for murexide-Zn and sulfate-Zn competitive systems.

A model of competitive complex formation between two ligands by a common metal ion based on equilibria presented in Eqs. (17)–(21), was used to create the artificial data for testing the applicability of the proposed method in such competitive equilibria. It has been simplified to make the data set relate to a real system. The characteristics of the model for creating the data set are given in Table 1. The method was tested by using the artificial data set and the calculated concentration profiles and equilibrium constants were exactly equal to those values used to create the model data. Thus, the proposed method is working quite well with regard to determining equilibrium concentration profiles and estimating the formation constant of colorless reaction. It is clear that the proposed method also can be used for study the competition of two metal ions for a particular ligand.

Competition of Zn^{2+} for complexation with sulfate ion and murexide at pH 5, is selected as an experimental model for evaluation of the proposed method. After selecting two appropriate wavelengths, considering the spectrum of murexide-Zn complex, the concentration profiles of free murexide were calculated in the presence and absence of sulfate ion. Stability constant of murexide-Zn complex was calculated in the absence of sulfate ion and by using the concentration profile of murexide calculated in the presence of sulfate ion and the mass balances of the system (Eqs. (19)–(21)), the concentration profiles of other chemical species (Zn , $Zn[Mu]$, SO_4^{2-} , and $ZnSO_4$) were calculated. Fig. 10 shows the concentration profiles obtained for a competitive system. The corresponding calculated formation constants are presented in Table 1.

5. Conclusion

A method based on combination of HPCIM and HPSAM represents a new approach to complete analysis of equilibrium systems studied by spectrophotometric mole ratio

method. Two successive stepwise conditional complex formation systems with three absorbing chemical components and nearly strong conditional stability constant, can be completely resolved by applying the proposed method. Also, the one step conditional complex formation systems with known stoichiometric ratio (n) and two absorbing chemical components which have strong spectral overlapping can be completely resolved, without considering the extent of conditional formation constant. The information that can be obtained is much more than that available through classical methods. The proposed method makes it possible to obtain the species concentration profiles in several ligand-metal ion complex formation systems by severe spectral overlapping. The method was tested with simulated data sets and reliability was obtained by reproducing the input formation constants and species concentration profiles. The method was also applied to experimental data in 1:1, 1:2, successive 1:1, and 1:2 metal ion to ligand complex formation and also competition of a metal ion for complexation with two ligands. The method presents advantages over the more traditional and some more complex chemometric methods based on its simplicity because the proposed method does not use the non-linear curve fitting and complex linear algebra and statistics. The proposed method in this paper was focussed to chemical systems with known chemical model, three light-absorbing chemical components, and overlapping of spectral profiles.

Appendix A. HPCIM for canceling the contribution of the ligand spectrum in the sample spectrum

H-Point curve isolation method can be used for obtaining the pure spectrum, which is sum of both complexes (ML and ML_2) spectra. We will use the term complex, instead of sum of both complexes for simplicity. After the selection of a reference wavelength from the spectrum of pure ligand

solution, the K_i is defined as:

$$K_i = \frac{l_{\text{ref}}}{l_i} \quad (\text{A.1})$$

where l_{ref} is the absorbance of pure ligand solution at reference wavelength. The ligand contribution to the signals can be canceled by subtracting $s_{\text{ref}} - K_i s_i$ to obtain

$$s_{\text{ref}} - K_i s_i = l_{\text{ref}} + c_{\text{ref}} - K_i(l_i + c_i) \quad (\text{A.2})$$

where s_{ref} is the absorbance of the mixture solution at the reference wavelength. Rearrangement gives

$$s_{\text{ref}} - K_i s_i = c_{\text{ref}} - K_i c_i \quad (\text{A.3})$$

As can be seen, the result is related only to the complex components. The later equation can be written in the following way

$$C_i = \frac{(s_{\text{ref}} - K_i s_i) - c_{\text{ref}}}{-K_i} = s_i - \frac{s_{\text{ref}} - c_{\text{ref}}}{K_i} \quad (\text{A.4})$$

From Eq. (A.4), it is easy to see that the entire spectrum of complex can be calculated by entering an appropriate value of c_{ref} , since every parameter in the equation is known except c_{ref} . Since it is only necessary to work with a pure ligand and mixture solution, the selection of the reference wavelength is arbitrary.

The next step is to estimate the c_{ref} value. The maximum of pure complex absorbance at the reference wavelength cannot be higher than the absorbance of the mixture solution which is analyzed at this wavelength, and the minimum value is the smallest absorbance value that provides non-negative absorbance value for the pure complex spectrum at any wavelength calculated from Eq. (A.4).

It is possible to plot several complex spectra from Eq. (A.4), considering the range described by the minimum and maximum absorbance values previously selected, and varying c_{ref} within this range to produce meaningful spectral differences between one spectrum and the other. The real complex spectrum will be one of all the calculated spectra.

To calculate the absorbance of the pure complex at the reference wavelength, it is necessary to find a pair of wavelengths with the same absorbance value in every hypothetical predicted spectrum, although there is difference in magnitude from one spectrum to the other. To ensure the accuracy of the prediction of the complex absorbance at the reference wavelength, the absorbance of the pure ligand solution at these wavelengths should be as different as possible. To facilitate the process of locating the wavelength pairs with the same value of absorbance for all predicted spectra, it is sufficient to check the maximum and minimum spectra, since they differ so much. If it is possible to find a pair of wavelengths with the same value of absorbance in these spectra, the absorbances at these wavelengths in the remaining spectra will also be equal. Having a set of $\lambda_m - \lambda_n$, for which $c_m = c_n$, there are three equations and three unknowns

$$\begin{aligned} s_{\text{ref}} - K_m s_m &= c_{\text{ref}} - K_m c_m \\ s_{\text{ref}} - K_n s_n &= c_{\text{ref}} - K_n c_n \quad c_m = c_n \end{aligned} \quad (\text{A.5})$$

So, the real value of c_{ref} can be calculated from

$$c_{\text{ref}} = s_{\text{ref}} - K_m s_m + \frac{K_m \{(s_{\text{ref}} - K_m s_m) - (s_{\text{ref}} - K_n s_n)\}}{K_n - K_m} \quad (\text{A.6})$$

Since this equation is only dependent on known quantities, it is then possible to estimate the complex component spectrum by using Eq. (A.4).

Appendix B. HPSAM for calculating the equilibrium concentrations of L and ML in two successive stepwise complex formations

In two step complex formation with absorbing species L, ML, and ML_2 , each solution at a particular mole ratio can be considered as a ternary mixture contains these compounds with concentrations [L], [ML], and $[\text{ML}_2]$, respectively. If this solution is measured spectrophotometrically, and for the spectra of the species the law of absorbance additivity is followed, then absorbance of the solution at wavelength j will be the sum of the individual absorbances of L, ML, and ML_2 at this wavelength:

$$A_j = \varepsilon_j^{\text{L}}[\text{L}] + \varepsilon_j^{\text{ML}}[\text{ML}] + \varepsilon_j^{\text{ML}_2}[\text{ML}_2] \quad (\text{B.1})$$

where A_j is the absorbance of the solution and ε_j^{L} , $\varepsilon_j^{\text{ML}}$, and $\varepsilon_j^{\text{ML}_2}$ are the absorption coefficients for L, ML, and ML_2 at the j th wavelength, respectively. Suppose that L is the analyte to be determined and ML and ML_2 are the interferents, then two wavelengths λ_1 and λ_2 , can be found where the following condition is fulfilled:

$$\frac{\varepsilon_1^{\text{ML}}}{\varepsilon_2^{\text{ML}}} = \frac{\varepsilon_1^{\text{ML}_2}}{\varepsilon_2^{\text{ML}_2}} = r_{\text{ML,ML}_2} \quad (\text{B.2})$$

where $r_{\text{ML,ML}_2}$ is the ratio factor. The couples of wavelengths that accomplish the stated condition are easily located by plotting a quotient between the spectra of both interferents.

If the absorbance values of a solution at each mole ratio at λ_1 and λ_2 are measured, and multiplying the absorbance measured at λ_2 by the ratio factor $r_{\text{ML,ML}_2}$, the following results are obtained:

$$A_1 = \varepsilon_1^{\text{L}}[\text{L}] + \varepsilon_1^{\text{ML}}[\text{ML}] + \varepsilon_1^{\text{ML}_2}[\text{ML}_2] \quad (\text{B.3})$$

$$\begin{aligned} r_{\text{ML,ML}_2} A_2 &= r_{\text{ML,ML}_2} \varepsilon_2^{\text{L}}[\text{L}] + r_{\text{ML,ML}_2} \varepsilon_2^{\text{ML}}[\text{ML}] \\ &\quad + r_{\text{ML,ML}_2} \varepsilon_2^{\text{ML}_2}[\text{ML}_2] \end{aligned} \quad (\text{B.4})$$

Simultaneous solution of these two equations permits the calculation of the concentration of the analyte [L], according to:

$$-C_{\text{H}} = [\text{L}] = \frac{A_1 - r_{\text{ML,ML}_2} A_2}{r_{\text{ML,ML}_2} \varepsilon_2^{\text{L}} - \varepsilon_1^{\text{L}}} \quad (\text{B.5})$$

where C_H is the unbiased analyte concentration in the solution, because Eq. (B.5) depends only on variables related to the analyte. It is clear that for calculating the r_{ML,ML_2} , only one spectrum of species ML and another of the species ML_2 are needed. Although this value depends on the concentration of ML and ML_2 , it will be equal at the two selected wavelengths, independent of their concentrations.

Concentration of species ML can be calculated in a similar way by calculating the appropriate r_{ML,ML_2} .

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